Applicants herewith provide a more legible Declaration. The Examiner has objected to the Figure legends in the Brief Description of Drawings at Page 10. Applicants have amended the description of Figures 8A and 8B in compliance with the Figure labeling requirements, pursuant to 37 C.F.R. §1.84(u)(1). The Examiner has also alleged that Figures 1-6, 9-11 and 16-17 do not meet the numbering requirements of 37 C.F.R. §1.84(u)(1). Applicants have amended the Brief Description of the Figures in compliance with the separate numbering requirements of 37 C.F.R. §1.84(u)(1). Withdrawal of the objection of the Brief Description of the Figures under 37 C.F.R. §1.84(u)(1) is therefore respectfully requested.

The title has been objected to as allegedly not descriptive. A new title has been provided which is consistent with the claimed subject matter.

Claims 44 and 45 have been rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite. Claims 44 and 45 have been rejected based on the recitation "hybridizes under low stringency conditions". In response, applicants have cancelled Claims 43-45 without prejudice and added Claims 50-54. Support for Claims 50-54 is also found throughout the specification and particularly at page 6, Lines 27-32, and Page 7, Lines 7-15, for example. No new matter has been added.

In addition and in an effort to further define the subject matter to which applicants are entitled, Claims 46-49 and 55-58 have been added. Claims 46-49 recite a process for the production of biologically active VEGF-B. Support for Claims 46-49 is found throughout the specification and particularly at original Claims 35, 36; page 7 lines 17-20; and Example 6. Claims 55-58 recite an isolated nucleic acid comprising the sequence of SEQ ID NO: 5 and the polypeptide sequence encoded thereby (SEQ ID NO: 6). Support for Claims

Applicants herewith provide a more legible Declaration. The Examiner has objected to the Figure legends in the Brief Description of Drawings at Page 10. Applicants have amended the description of Figures 8A and 8B in compliance with the Figure labeling requirements, pursuant to 37 C.F.R. §1.84(u)(1). The Examiner has also alleged that Figures 1-6, 9-11 and 16-17 do not meet the numbering requirements of 37 C.F.R. §1.84(u)(1). Applicants have amended the Brief Description of the Figures in compliance with the separate numbering requirements of 37 C.F.R. §1.84(u)(1). Withdrawal of the objection of the Brief Description of the Figures under 37 C.F.R. §1.84(u)(1) is therefore respectfully requested.

The title has been objected to as allegedly not descriptive. A new title has been provided which is consistent with the claimed subject matter.

Claims 44 and 45 have been rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite. Claims 44 and 45 have been rejected based on the recitation "hybridizes under low stringency conditions". In response, applicants have cancelled Claims 43-45 without prejudice and added Claims 50-54. Support for Claims 50-54 is also found throughout the specification and particularly at page 6, Lines 27-32, and Page 7, Lines 7-15, for example. No new matter has been added.

In addition and in an effort to further define the subject matter to which applicants are entitled, Claims 46-49 and 55-58 have been added. Claims 46-49 recite a process for the production of biologically active VEGF-B. Support for Claims 46-49 is found throughout the specification and particularly at original Claims 35, 36; page 7 lines 17-20; and Example 6. Claims 55-58 recite an isolated nucleic acid comprising the sequence of SEQ ID NO: 5 and the polypeptide sequence encoded thereby (SEQ ID NO: 6). Support for Claims

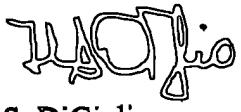
55-58 is found throughout the specificati n and particularly, at original Claim 29; Page 7, lines 17-20; Figures 5 and 6; and Table I, Page 14. Applicants have also amended the claims to properly recite VEGF-B in conformance with current standard biological nomenclature. No new matter has been added.

Accordingly, in view of the foregoing amendments, the rejections of Claims 44 and 45 under 35 U.S.C. §112, second paragraph, are overcome and withdrawal thereof is respectfully requested.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

Thus, in view of the foregoing amendments and remarks, it is respectfully submitted that the present application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,



Frank S. DiGiglio
Registration No. 31,346

SCULLY, SCOTT, MURPHY & PRESSER
400 Garden City Plaza
Garden City, New York 11530
(516) 742-4343

FSD:PIB:lf/bg

Version with markings to show changes made

In the title:

Please replace the title with the following new title:

[A NOVEL GROWTH FACTOR AND A GENETIC SEQUENCE ENCODING SAME]A VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) MOLECULE AND PROCESS FOR PRODUCING SAME

In the specification:

Paragraph beginning at page 7, line 22, has been amended as follows:

The present invention is further directed to the murine homologue of human VEGF(referred to herein as "mVRF"). The mVRF has approximately 85% identity and 92% conservation of amino acid residues over the entire coding region compared to human VEGF. The mVRF is encoded by a nucleic acid molecule comprising a nucleotide sequence substantially as set forth in [Figure 9] Figures 9A-9D.

Paragraph beginning at page 9, line 16, has been amended as follows:

[Figure 1 Nucleotide] Figures 1A-1D show the nucleotide sequence [SEQ ID NO:1] (SEQ ID NO:1)and corresponding amino acid sequence [SEQ ID NO:2] (SEQ ID NO:2) of $VEGF_{165}$.

Paragraph beginning at page 9, line 19, has been amended as follows:

[Figure 2 Nucleotide] Figures 2A-2F show the nucleotide sequence [SEQ ID NO:3] (SEQ ID NO:3) and corresponding amino acid sequence [SEQ ID NO:4] (SEQ ID NO:4) of SOM175.

Paragraph beginning at page 9, line 22, has been amended as follows:

[Figure 3 Results] Figures 3A-3B show the results of BLAST search with SOM175 protein sequence.

Paragraph beginning at page 9, line 24, has been amended as follows:

[Figure 4] Figures 4A-4D show the BESTFIT alignment of VEGF cDNA and SOM175 cDNA.

Paragraph beginning at page 9, line 26, has been amended as follows:

[Figure 5 Multiple] Figures 5A-5F show the multiple alignment of $VEGF_{165}$ with SOM175 and its splice variants at the nucleotide level.

Paragraph beginning at page 9, line 29, has been amended as follows:

[Figure 6 Multiple] Figures 6A-6C show the multiple alignment of $VEGF_{165}$ with SOM175 and its splice variants at the amino acid level.

Paragraph beginning at page 10, line 7, has been amended as follows:

[Figure 9 Nucleotide] Figures 9A-9D show the nucleotide and predicted peptide sequences derived from mVRF cDNA clones. Numbering of nucleotides are given on the left, starting with the A of the initiation codon. Amino acids are numbered on the right, starting from the first residue of the predicted mature protein after the putative signal peptide has been removed. The alternatively spliced region is double underlined and the resulting peptide sequence from each mRNA is included. A potential polyadenylation signal is indicated in boldface. Start and stop codons of $mVRF_{167}$ and $mVRF_{186}$ are underlined and a polymorphic AC repeat in the 3' UTR is indicated by a stippled box. The positions of intron/exons boundaries are indicated by arrowheads.

Paragraph beginning at page 10, line 17, has been amended as follows:

[Figure 10] Figures 10A-10B show the BESTFIT alignments of human and murine VRF protein isoforms. A: $mVRF_{167}$ and $hVRF_{167}$. B: $mVRF_{186}$ and $hVRF_{186}$ from the point where the sequences diverge form the respective 167 amino acid isoforms. Amino acid identities are marked with vertical bars and conserved amino acids with colons. An arrow marks the predicted signal peptide cleavage site of human and mouse VRF.

Paragraph beginning at page 10, line 23, has been amended as follows:

[Figure 11] Figures 11A-11B show the BESTFIT alignment of $mVRF_{167}$ and $mVEGF_{188}$ (Brier et al., 1992) peptide sequences. An arrow marks the signal peptide cleavage site of mVEGF. Identical amino acids are indicated by vertical bars and conservative substitutions by colons. Numbering of amino acids is as described in the legend to Figure 9.

Paragraph beginning at page 11, line 8, has been amended as follows:

[Figure 14 Film] Figures 14A-14E show film autoradiographs (A-C) and dark-field micrographs (D-E) illustrating the expression pattern of mVRF and mRNA in the mouse. In the E14 mouse embryo (A) positive signals are present over the developing heart (Ha) and cerebral cortex (Cx). A low background signal is also present over other tissues in the section. In the E17 embryo (B) and the heart (Ha) is clearly visible due to a strong hybridisation signal. An equally strong signal is present over brown adipose tissue (Fa) in the back and around the thoracic cage. A moderate hybridisation signal is present over the spinal cord (SC) and the tongue (T). The background signal is reduced compared with the E14 embryo. In the young adult mouse (C-D), positive signals are present over the heart (Ha) and adipose tissue (Fa) around the thoracic cage, while, for example, the lungs (Lu) are unlabeled[)]. The hybridisation signal over the heart is evenly distributed over the entire left ventricle, including papillary muscles (D). In the E17 heart hybridised with an excess of cold probe, no positive signal is present (E). Scale bars = 0.5 mm (A), 1.2 mm (B), 1 mm (C), 0.3 mm (D), 0.1 mm (E).

Paragraph beginning at page 11, line 23, has been amended as follows:

[Figure 15 Dark]- Figures 15A-15D show dark – (A and C) and bright-field (B and D) micrographs showing mVRF mRNA expression in mouse adipose tissue (A-B) and spinal cord (C-D). A strong hybridisation signal is present over fat (A), as shown by the strong labeling in Sudan black stained sections (B). A weak signal is present also in skeletal muscle

(in A-B). In the adult spinal cord (C) the mVRF probes gave a neuronal staining pattern over the gray matter. Toloudine counterstaining showing that motoneurons in the ventral horn (D), interneurons in the deep part of the dorsal horn and around the central canal (not shown) where largely positive for mVRF mRNA. Scale bars = 0.1mm (A), 0.1 mm (B), 0.25 mm (C), 0.015 mm (D).

Paragraph beginning at page 12, line 1, has been amended as follows:

[Figure 16 Effect of VEGF on embryonic day 8 (E8) chick sensory neurons as determined by % survival, % neurite outgrowth and average neurite length (μm)] Figures 16A-16C show the effect of VEGF on embryonic day 8 (E8) chick sensory neurons as determined by % survival (Fig. 16A), % neurite outgrowth (Fig. 16B) and average neurite length (μm) (Fig. 16C).

Paragraph beginning at page 12, line 4, has been amended as follows:

[Figure 17 Effects of VEGF and SOM175 on chick glia. Tested were CNS glial, peripheral glia and CNS oligodendrocytes] Figures 17A-17C show the effects of VEGF and SOM175 on chick glia. Tested were CNS glial (Fig. 17A), peripheral glia (Fig. 17B) and CNS oligodendrocytes (Fig. 17C).

Paragraph beginning at page 15, line 18, has been amended as follows:

The entire sequence of the cDNA clone (SOM175) was compiled and is shown in [Figure 2] Figures 2A-2F with its corresponding amino acid sequence. This sequence was

screened for open reading frames using the MAP program (GCG, University of Wisconsin). A single open reading frame of 672bp was observed (see [Figure 2] Figures 2A-2F). There appears to be little 5' untranslated sequences (2bp). The 3' untranslated region appears to be complete as it includes a poly-adenylation signal poly-A tail.

Paragraph beginning at page 15, line 25, has been amended as follows:

Database homology searches were performed using the BLAST algorithm (run at NCBI, USA). This analysis revealed homology to several mammalian forms of VEGF (see [Figure 3] Figures 3A-3B). The amount of homology between SOM175 and human $VEGF_{165}$ was determined using the BESTFIT program (GCG, University of Wisconsin; see Figures [4 and 5] 4A-4D and 5A-5F). Nucleotide homology was estimated at 69.7% and protein homology was estimated as at least 33.3% identity and 52.5% conservation using BESTFIT analysis. BLAST analysis on nucleotide sequences revealed the almost complete match to a human expressed sequence tag EST06302 (Adams et al., 1993).

Paragraph beginning at page 16, line 1, has been amended as follows:

These data indicate that SOM175 encodes a growth factor that has structural similarities to VEGF. Both genes show start and stop codons in similar positions and share discrete blocks of homology. All 8 cysteines as well as a number of other VEGF residues believed to be involved in dimerisation are conserved. These residues are Cysteine-47, Proline-70, Cysteine-72, Valine-74, Arginine-77, Cysteine-78, Glycine-80, Cysteine-81, Cysteine-82, Cysteine-89, Proline-91, Cysteine-122 and Cysteine-124 and are shown in

[Figure 6] Figures 6A-6C. Given the structural conservation between VEGF and the SOM175 gene product it is also possible that they share functional similarities. It is proposed that SOM175 encodes a VEGF-like molecule that shares some properties with VEGF but has unique properties of its own. The nucleotide sequence and corresponding amino acid sequence of $VEGF_{165}$ is shown in [Figure 1] Figures 1A-1D.

Paragraph beginning at page 16, line 14, has been amended as follows:

The percentage similarity and divergence between $VEGF_{165}$ family and SOM175 family (protein) were analysed using the Clustal method, MegAlign Software, DNASTAR, Wisconsin. The results are shown in Tables 2.1 and 2.2. The alternatively spliced forms of SOM175 are abreviated to SOM715-e6 where all of exon 6 is deleted; SOM715-e6 and 7 where all of exons 6 and 7 are deleted; and SOM175-e4 where all of exon 4 is deleted. The spliced form of SOM175 are shown in Figure 7. Genomic maps of SOM175 showing intron/exon boundaries are shown in [Figure 8a and 8b] Figures 8A and 8B.

Paragraph beginning at page 24, line 23, has been amended as follows:

Murine VRF homologues were isolated by screening a murine cDNA library with an hVRF cDNA clone. Five clones of sizes varying from 0.8-1.5 kb were recovered and sequenced. The cDNA sequences were [complied] compiled to give a full length 1041 bp cDNA sequence covering the entire open reading frame (621 bp or 564 bp depending on the

splice form, see below) and 3' UTR (379 bp), as well as 163 bp of the 5' UTR ([Figure9] Figures 9A-9D).

Paragraph beginning at Page 25, line 3, has been amended as follows:

The predicted N-terminal signal peptide of hVRF appears to be present in mVRF with 81% identity (17/21 amino acids). Peptide cleavage with mVRF is expected to occur after reside 21 [(Figure 10)] (Figures 10A-10B). These data suggest that mature mVRF is secreted and could therefore conceivably function as a growth factor.

Paragraph beginning at Page 25, line 8, has been amended as follows:

As with hVRF, two open reading frames (ORFs) were detected in cDNAs isolated by library screening. Four of five clones were found to be alternatively spliced and lacked a 101 bp fragment homologous to exon 6 of hVRF. The predicted peptide sequences of the two isoforms of mVRF were determined and aligned with the corresponding human isoforms [(Figure 10)] (Figures 10A-10B).

Paragraph beginning at Page 25, line 14, has been amended as follows:

The message encoding $mVRF_{186}$ contains a 621 bp ORF with coding sequences terminating at position +622, towards the end of exon 7 [(Figure 9)] (Figures 9A-9D). The smaller message encoding $mVRF_{167}$ actually terminates downstream of the +622 TAG site due to a frame shift resulting from splicing out of the 101 bp exon 6 and the introduction of a

stop codon (TGA) at position +666, near the beginning of exon 8 [(Figure 9A-9D)] (Figures 9A-9D).

Paragraph beginning at Page 25, line 21, has been amended as follows:

The $mVRF_{186}$ protein has strong homology to the amino and central portions of VEGF while the carboxyl end is completely divergent [an] and is alanine rich. $mVRF_{167}$ possesses these similarities and also maintains homology to mVEGF right through to the C-terminus [(Figure 11)] (Figures 11A-11B). The overall homology of $mVRF_{167}$ to $hVRF_{167}$ was 85% identity and 92% similarity, respectively [(Figures 10)] (Figures 10A-10B). Likewise, homology between $mVRF_{167}$ and mVEGF (Breier, et al. 1992) was 49% identity and 71% conservative amino acid substitution, respective [(Figure 11)] (Figures 11A-11B).

Paragraph beginning at Page 25, line 29, has been amended as follows:

A canonical vertebrate polyadenylation signal (AATAAA) (Birnstiel, et al., 1986) was not present in the mVRF cDNA, however, the closely matching sequence GATAAA is present at similar positions in both mouse and human VRF cDNAs [(Figure 9)] (Figures 9A-9D). In contrast to hVRF, mVRF was found to contain an AC dinucleotide repeat at the extreme 3' end of the 3'UTR (nucleotide positions 998 to 1011, [(Figure 9)] Figures 9A-9D). Polymorphism of this repeat region was observed between some of the mVRF cDNAs, with the number of dinucleotides varying from 7 to 11.

Paragraph beginning at Page 26, line 17, has been amended as follows:

Exons 6 and 7 are contiguous in mVRF, as has been found to occur in the human homologue. The strong sequence homology between exon 6 of mVRF and hVRF [(Figure 10] (Figures 10A-10B) suggests that this sequence is not a retained intronic sequence but rather encodes a functional part of the VRF_{186} isoform.

Paragraph beginning at Page 27, line 2, has been amended as follows:

Northern analysis of RNA from adult mouse tissues (muscle, heart, lung and liver) showed that expression appears to be ubiguitous and occurs primarily as a major band of approximately 1.3kb in size [(Figure 14)] (Figures 14A-14E). This is somewhat different to the pattern observed for hVRF in which two major bands of 2.0 and 5.5 kb have been identified in all tissues examined. The 1.3 kb murine message presumably corresponds to the shorter of the human transcripts and the size variation thereof is most likely due to a different in the length of the respective 5' UTRs.

Paragraph beginning at Page 29, line 25, has been amended as follows:

The results are shown in [Figure 16] Figures 16A-16C. The results show that VEGF is effective in promoting neuronal survival but that this requires the presence of glial cells. [Figure 17] Figures 17A-17C shows the results of the effect of VEGF and SOM175 on three types of chick glia. The glia tested were CNS glia (Figure 17A), peripheral glia (Figure 17B) and CNS oligodendrocytes (Figure 17C). Heparin was used at 10 μg/ml in all cultures

and the assay was read at 24 hours. Results were measured in ^{3}H-thymidine counts using 2000 cells per well.

Paragraph beginning at Page 31, line 7, has been amended as follows:

Greatest activity was seen with preparations of SOM175 absent exon 6 (SOM$_{\Delta}$X6) on mouse astroglial cell cultures, where there was a significant stimulus to their proliferation when delivered in conjunction with heparin [(Figures 16)] (Figures 16A-16C). Little stimulus was given to the proliferation of oligodendroglial cells [(Figure 17)] (Figures 17A-17C), and very little discernable potentiation of the survival response of isolated forebrain neurons (Figure 18). The standard deviation on all three graphs for each point was less than 8%.

IN THE CLAIMS:

Please cancel Claims 43-45 without prejudice.

Please add the following new Claims:

46. (New) A process for the production of biologically active VEGF-B, said method comprising expressing a nucleic acid molecule of SEQ ID NO: 3 in a host and isolating said VEGF-B.

47. (New) A process for the production of a biologically active VEGF-B, said method comprising expressing a nucleic acid molecule of SEQ ID NO: 5 in a host and isolating said VEGF-B.

48. (New) A process for the production of a biologically active VEGF-B, said method comprising expressing a nucleic acid molecule of SEQ ID NO: 7 in a host and

isolating said VEGF-B.

49. (New) A process for the production of a biologically active VEGF-B, said method comprising expressing a nucleic acid molecule of SEQ ID NO: 9 in a host and isolating said VEGF-B.

50. (New) A process for the production of biologically active VEGF-B, said method comprising expressing a nucleic acid molecule which hybridizes under high stringency conditions to a nucleic acid of SEQ ID NOS: 3, 5, 7 and 9 in a host and isolating said VEGF-B, wherein said high stringency conditions comprise 0.1-1X SSC/0.1% w/v SDS at 60°C for 1-3 hours.

51. (New) A process for the production of biologically active VEGF-B, said method comprising expressing a nucleic acid molecule which hybridizes under high stringency conditions to a nucleic acid of SEQ ID NO:3 in a host and isolating said VEGF-B, wherein said high stringency conditions comprise 0.1-1X SSC/0.1% w/v SDS at 60°C for 1-3 hours.

52. (New) A process for the production of biologically active VEGF-B, said method comprising expressing a nucleic acid molecule which hybridizes under high stringency conditions to a nucleic acid of SEQ ID NO:5 in a host and isolating said VEGF-B, wherein said high stringency conditions comprise 0.1-1X SSC/0.1% w/v SDS at 60°C for 1-3 hours.

53. (New) A process for the production of biologically active VEGF-B, said method comprising expressing a nucleic acid of SEQ ID NO:7 in a host and isolating said VEGF-B, wherein said high stringency conditions comprise 0.1-1X SSC/0.1% w/v SDS at

60°C for 1-3 hours.

54. (New) A process for the production of biologically active VEGF-B, said method comprising expressing a nucleic acid molecule which hybridizes under high stringency conditions to a nucleic acid of SEQ ID NO:9 in a host and isolating said VEGF-B, wherein said high stringency conditions comprise 0.1-1X SSC/0.1% w/v SDS at 60°C for 1-3 hours.

55. (New) The process according to any one of Claims 43, 46, 50 or 51 wherein said VEGF-B is human VEGF-B.

56. (New) An isolated nucleic acid comprising the sequence of SEQ ID NO:5.

57. (New) An isolated nucleic acid encoding or complementary to a nucleic acid encoding a polypeptide consisting of the sequence of SEQ ID NO:6.

58. (New) An isolated nucleic acid consisting of the sequence of SEQ ID NO:5.